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EXAMINER	
FERNANDEZ, SUSAN EMILY	
ART UNIT	**PAPER NUMBER**
1651	

DATE MAILED: 03/11/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/669,382	HUANG ET AL.	
	Examiner	Art Unit	
	Susan E. Fernandez	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-14 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-14 is/are rejected.

7)☒ Claim(s) 9 is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date 2-4-04.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claims 1-14 are pending and are examined on the merits.

Claim Objections

Claim 9 is objected to because of the following informalities: Claim 9 does not define "DMSO". The full name, "dimethyl sulfoxide," should be given at the first instance the abbreviation appears in the claims. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 2 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for performing an end point assay for protein disulfide isomerase (PDI) activity wherein a substrate for PDI activity is insulin and HIV gp120, does not reasonably provide enablement for such a method wherein the substrate is prolyl 4-hydroxylase or hypoxia-inducible factor. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to perform the invention commensurate in scope with these claims.

Regarding undue experimentation, *In re Wands*, 8 USPQ2d 1400, at 1404 (Fed. Cir. 1988) states:

> Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in *Ex parte Forman*. They include (1) the quantity of

> experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. (Citations omitted).

Claim 1 recites that the reaction mixture comprising of a substrate for PDI activity is incubated for "a period of time sufficient to reduce disulfide bonds present in said substrate". However, this is unsubstantiated by the consensus of the art for prolyl 4-hydroxylase and hypoxia-inducible factor.

According to Noiva (Seminars in Cell & Developmental Biology, 1999, 10: 481-493), PDI is the β-subunit of prolyl 4-hydroxylase (P4H), acting as a chaperone to the α-subunit of P4H (page 485, "Multifunctionality" section). Furthermore, there is evidence that there is "cooperativity of substrate binding" between the two subunits and that PDI serves to assist with "subcellular localization of the P4H enzyme to the lumen of the rough ER" (page 485, second column, second paragraph). No evidence is provided there is a reduction of disulfide bonds in P4H once P4H comes in contact with PDI.

Graven et al. (Am. J. Physiol. Lung Cell. Mol. Physiol., 2002, 282: L996-L1003) reviews the connection between PDI and hypoxia-inducible factor-α (HIF-α). See page L1001. Proline hydroxylation is essential for HIF-α regulation. Referring to the pathway for HIF-α regulation, Graven et al. states that "the role of PDI upregulation in this hypoxiasensing pathway is unclear" (page L1001, second column, first paragraph). No indication is given that HIF-α is a substrate of PDI activity wherein disulfide bonds are reduced when reacted with PDI.

Considering that the state of the prior art and the specification do not provide evidence to the contrary, it is unlikely that the person of ordinary skill in the art would have a reasonable expectation of success in performing the assay wherein PDI causes reduction of disulfide bonds

in P4H and hypoxia-inducible factor. Taken in light of the teachings of Noiva and Graven et al. and the limited guidance provided by the applicants in the specification, the skilled artisan would not have been lead to any conclusion regarding the relationship of P4H and hypoxia-inducible factor as substrates of PDI activity through reduction of disulfide bonds.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-14 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is indefinite because the step of measuring the optical density is unclear and broad. It is not clear whether the optical density is measured only once or at several time points. What optical densities are compared in order to judge the presence of PDI activity? Furthermore, claim 1 recites "incubating said reaction" though there is no mention of a reaction earlier in the claim. Thus claim 1 and dependent claims 2-14 are rejected under 35 U.S.C. 112, second paragraph.

Claims 9 and 13 recites "said reaction **mix**" whereas parent claim 1 recites a "reaction mixture". Thus claims 9 and 13 and dependent claim 14 are rejected under 35 U.S.C. 112, second paragraph.

The phrase "defined source of PDI activity" renders claim 13 indefinite because it is unclear what would differentiate a "defined source" from any other source of PDI activity. For examination purposes, addition of a "defined source of PDI activity" would be satisfied by the

presence of a source of PDI activity in the reaction mixture as recited in claim 1. Thus claim 13 and dependent claim 14 are rejected under 35 U.S.C. 112, second paragraph.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-9 and 12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bonfils (Eur. J. Biochem., 1998, 254: 420-427) in view of Qvist et al. (U.S. Pat. 6,110,689), Cahoon et al. (WO 00/22100), and Moussebois et al. (U.S. Pat. 4,397,960).

Bonfils discloses a method of measuring PDI activity by measuring disulfide reduction of insulin. See page 422, first paragraph under "PDI activities". The reaction mixture used in

Bonfils included 0.05-0.4 μM of pure bovine liver PDI as the source of PDI activity, 0.16 M insulin as the substrate for PDI activity, and dithiothreitol (DTT) as the reducing agent. According to Calbiochem (http://www.emdbiosciences.com/product/539425), the molecular weight of bovine liver PDI is 107,000 g/mol, thus 0.05 μM bovine liver PDI is approximately equal to 5 μg/mL, and is considered a "defined source of PDI activity". Bonfils states that 1 mM DTT is used in the reaction mixture (page 424, first column, second paragraph). Furthermore, the optical density is measured at 650 nm, and the readings are shown on Figure 5, page 424, where the reaction mixture consisting of PDI is incubated for up to 20 minutes.

Bonfils does not expressly disclose stopping the reaction with hydrogen peroxide.

Qvist et al. discloses an assay wherein an enzyme reaction is run in an automated microtiter plate reader and stopped prior to obtaining an optical density reading. See column 12, lines 47-50.

Cahoon et al. discloses that PDI "catalyzes the rearrangement of both intrachain and interchain disulfide bonds in proteins to form native structures" and that "PDI needs reducing agents or partly-reduced enzyme". See page 1, lines 20-25.

Moussebois et al. discloses that "any DTT can be inactivated by oxidation with hydrogen peroxide" (column 3, lines 11-12).

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to stop the reaction used in Bonfils with hydrogen peroxide when DTT is the reducing agent, particularly if the reaction is run in an automated microtiter plate reader. Furthermore, the selection of a suitable final concentration of hydrogen peroxide added to stop

the reaction would have been a routine matter of optimizing a result-effective parameter at the time of applicant's invention.

One of ordinary skill in the art would have been motivated to do this because stopping the reaction at specific time points would allow for reaction mixture samples to be saved for future optical density readings. This would allow for greater efficiency in experimentation, allowing one to carry out several PDI activity assays at once. As Qvist shows, multiple assays can be carried out in a microtiter plate, wherein halting the enzyme reaction occurs prior to performing optical density readings. Cahoon et al. discloses that a reducing agent is required for PDI activity on disulfide bonds, thus it would have been obvious to introduce an agent that would act on the reducing agent in order to halt PDI activity, which would stop the reaction between PDI and the substrate. A holding of obviousness is therefore clearly required.

Claims 1-9 and 12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bonfils, Qvist et al., Cahoon et al., and Moussebois et al. as applied to claims 1-9 and 12 above, and further in view of Budavari (Merck Index, 12th edition, Merck & Co., Inc., 1996, page 551).

As discussed above, Bonfils, Qvist et al., Cahoon et al., and Moussebois et al. render claims 1-8 and 12 obvious.

These references do not expressly disclose including less than 2% DMSO to the reaction mixture used in the assay for PDI activity.

Budavari discloses that dimethyl sulfoxide (DMSO) is soluble in water, ethanol, acetone, ether, benzene, and chloroform. Furthermore, it is listed as a solvent of various compounds.

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to have included DMSO in the reaction mixture used in Budavari et al. Furthermore, the selection of a suitable final concentration of DMSO in the reaction mixture would have been a routine matter of optimizing a result-effective parameter at the time of applicant's invention.

One of ordinary skill in the art would have been motivated to do this because addition of DMSO, a solvent, would ensure that all ingredients in the reaction mixture would have been in solution. A holding of obviousness is therefore clearly required.

Claims 1-9 and 12-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bonfils, Qvist et al., Cahoon et al., and Moussebois et al. as applied to claims 1-9 and 12 above, and further in view of Ryser (WO 94/04185).

As discussed above, Bonfils, Qvist et al., Cahoon et al., and Moussebois et al. render claims 1-9 and 12 obvious.

These references do not expressly disclose including HIV gp120 as the substrate for PDI activity in the reaction mixture. Furthermore, they do not expressly disclose a reaction mixture further comprising a sample comprising a candidate PDI modulating agent.

Ryser discloses an invention which demonstrates that when PDI is inhibited, entry of HIV and other viruses is completely or partially reduced, thus "the adverse effects of infection are also reduced" (page 4, lines 19-24). PDI inhibition also reduces or completely inhibits the passage of a toxin, such as diphtheria, across a membrane, thus reducing cytotoxicity (page 4, lines 3-7).

Ryser has investigated the role of "cleavage of critical disulfide bonds in the outer proteins (envelope proteins) of HIV" in HIV infection and that inhibition of such bonds "with DTNB or other membrane impermeant sulfhydryl blockers might inhibit HIV infection of human cells" (page 20, lines 28-34). DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) inhibits PDI directly (page 10, lines 30-33). Ryser discusses HIV gp120 and discloses that "cleavage of specific disulfides of gp120 can be expected to cause conformational changes leading to and required for virus penetration and infection of human cells" (page 21, lines 11-14).

Ryser discusses experiments that assess the effect of various compounds on PDI function, and their relation to toxins and HIV gp120. Moreover, example 6 describes the inhibition of PDI activity by bacitracin, wherein a glutathione:insulin transhydrogenase assay is used involving addition of an agent to stop the reaction for activity determination (page 51, particularly lines 21-29).

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to substitute insulin used in Bonfils for HIV gp120 as the substrate for PDI activity. Furthermore, it would have been obvious to have included a candidate PDI modulating agent in the reaction mixture as described in Bonfils, such as a PDI inhibitor.

One of ordinary skill in the art would have been motivated to do this because HIV gp120 consists of disulfide bonds which can be acted on by PDI. The spread of HIV is a significant and critical global issue, and many resources are being invested for prevention and treatment of its infection. Because of the potential use of PDI inhibitors as drugs for treating/preventing HIV and reduction of cytotoxicity, one would have been motivated to assess potential PDI modulators for their effect on PDI activity. This drug screening would have been accomplished by

performing a PDI activity assay wherein a potential PDI modulator is included in the reaction mixture. A holding of obviousness is therefore clearly required.

Claims 1-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bonfils, Qvist et al., Cahoon et al., Moussebois et al., and Ryser as applied to claims 1-9 and 12-14 above, and further in view of Dunlay et al. (U.S. Pat. 5,989,835).

As discussed above, Bonfils, Qvist et al., Cahoon et al., Moussebois et al., and Ryser render claims 1-9 and 12-14 obvious.

These references do not teach performing a plurality of PDI activity assays in parallel, or performing these assays in a microtiter plate.

Dunlay et al. provides background information on drug discovery, including high throughput screening of compound libraries using a particular assay based on a specific disease target. See column 1, lines 9-31. High throughput screening involves "parallel handling" of many compounds, use of microtiter plates, and measurement of optical density from each well in a microtiter plate.

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to perform multiple PDI activity assays as disclosed in Bonfils, Qvist et al., Cahoon et al., Moussebois et al., and Ryser in a microtiter plate.

One of ordinary skill in the art would have been motivated to do this because it would have allowed for more rapid screening of candidate PDI modulating agents. As was discussed above, PDI inhibitors would have served as drugs for treating/preventing HIV infections and reduction of cytotoxicity. Thus it would have been obvious to use typical methods used in drug

discovery with the PDI activity assay for determining HIV and cytotoxicity drug candidates. A holding of obviousness is therefore required.

Claims 1-9 and 12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bonfils, Qvist et al., Cahoon et al., and Moussebois et al. as applied to claims 1-9 and 12 above, and further in view of Myllyla et al. (European Journal of Biochemistry, 1983, 134(1): 7-11).

As discussed above, Bonfils, Calbiochem, Qvist et al., Cahoon et al., and Moussebois et al. render claims 1-9 and 12 obvious.

These references only offer bovine liver PDI as a source of PDI activity. This source is considered a "biological sample".

Moussebois et al. provides further support of using a biological sample as a source of PDI activity included in the reaction mixture used for the PDI activity assay. See abstract. The Moussebois study assessed PDI activity in chick embryo tendon, cartilage cells, confluent cultures of human skin, lung fibroblasts, and mouse 3T6 fibroblasts. All these biological samples had PDI activity.

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to use the biological samples listed in Mouseebois et al. as substrates for PDI activity in the reaction mixture disclosed by Bonfils.

One of ordinary skill in the art would have been motivated to do this because there would have been a reasonable expectation of success in carrying out a PDI activity assay with any source of PDI activity, regardless of whether the PDI source purely consists of PDI. Since PDI activity in the biological samples had been successfully assessed in the Mouseebois study, there

would have been a reasonable expectation of success in determining PDI activity using the methods taught by Bonfils, Qvist et al., Cahoon et al., and Moussebois et al. A holding of obviousness is clearly required.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Susan E. Fernandez whose telephone number is (571) 272-3444. The examiner can normally be reached on Mon-Fri 8:30 am - 5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Wityshyn can be reached on (571) 272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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